Extraction, Isolation and Validation of Amide Alkaloids from Natural sources

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Introduction

Alkaloids are organic chemical substances with simple nitrogen atoms that are found in nature. The name is derived from the word "alkaline," which was once used to refer to any base that contained nitrogen. Alkaloids are a class of natural chemicals that are produced by a wide range of species, including bacteria, fungi, plants, and mammals (also called secondary metabolites). Plant alkaloids are mostly found in the four plant groups Asteraceae, Apocynaceae, Boraginaceae, and Fabaceae.

This large class of over 12,000 different natural chemicals is referred to as alkaloids. A basic nitrogen atom must be present in any location of the molecule for it to be classified as an alkaloid; this does not include nitrogen found in amide or peptide bonds. Alkaloids are a class of compounds with a wide range of structural and biological diversity and significant pharmacological effects.For example, plant alkaloids include the narcotic analgesics, morphine andcodeine, apomorphine (a derivative of morphine) used in Parkinson's disease, the muscle relaxant papaverine, and theantimicrobial agents sanguinarine and berberine. [1,2].

The term alkaloid was introduced by W. Meisner at the beginning of the nineteenth century to designate natural substances reacting like bases, in other words like alkalis (from the Arabic alkali, soda, and from the Greek eidos,appearance) [3].

Although plants containing alkaloids have been used by man for at least 3000 years as medicines, teas, and potions, the compounds responsible for activity were not isolated and characterized until the 19th century. Many proved to be challenging chemical problems and the structures were resolved only in recent years [4]. The first isolations of alkaloids in the nineteenth century followed their introduction into medicine of a number of alkaloid-containing drugs and were coincidental with the advent of the percolation process for the extraction of drugs.

Typically, this nitrogen is basic, included in a heterocyclic ring, and derives from an amino ac id.

Analkaloid is a cyclic molecule with nitrogen in a negative oxidation state that is only found i n a small number of living things, according to Pelletier (1983).

Although some have modest effects, alkaloids virtually invariably have physiological activity in animals [4]. The majority of alkaloids are distinct crystals that combine with acids to generate salts. They might be present in the plant in their free form, as salts, or as N-oxides. The majority of alkaloids also contain oxygen along with the atoms carbon, hydrogen, and nitrogen. Some of them are liquids without oxygen, as conine from hemlock and nicotine from tobacco.

Although color alkaloids are uncommon, they are not unheard of; for instance, berberine is yellow and sanguinarine salts are copper-red[5].

One of the most interesting and significant areas of bioorganic chemistry is the chemistry of alkaloids. Initially, N-containing substances of plant origin with a strongly basic character were referred to as "alkaloids. Alkaloids are a diverse category of chemical molecules with natural nitrogen generated by fungus, micro- and marine organisms, and plants. Alkaloids are exceedingly varied because, in contrast to other types of natural substances, their structural frameworks are almost limitless and they contain a N atom in their molecules[6, 7]. They are categorized using a variety of signatures, including those from chemical or natural sources.

The distribution of alkaloids according to their primary structure, the major C-N skeleton, is the most accurate and widely used categorization of alkaloids. Alkaloids are categorized into the following significant groups, (Fig. 1) in accordance with the last signature, including pyrrolidine, pyridine, quinoline, isoquinoline, indole, quinazoline, steroidal, diterpenoid, and other alkaloids. These groups are grouped into a number of subgroups based on the structural characteristics of their representatives.

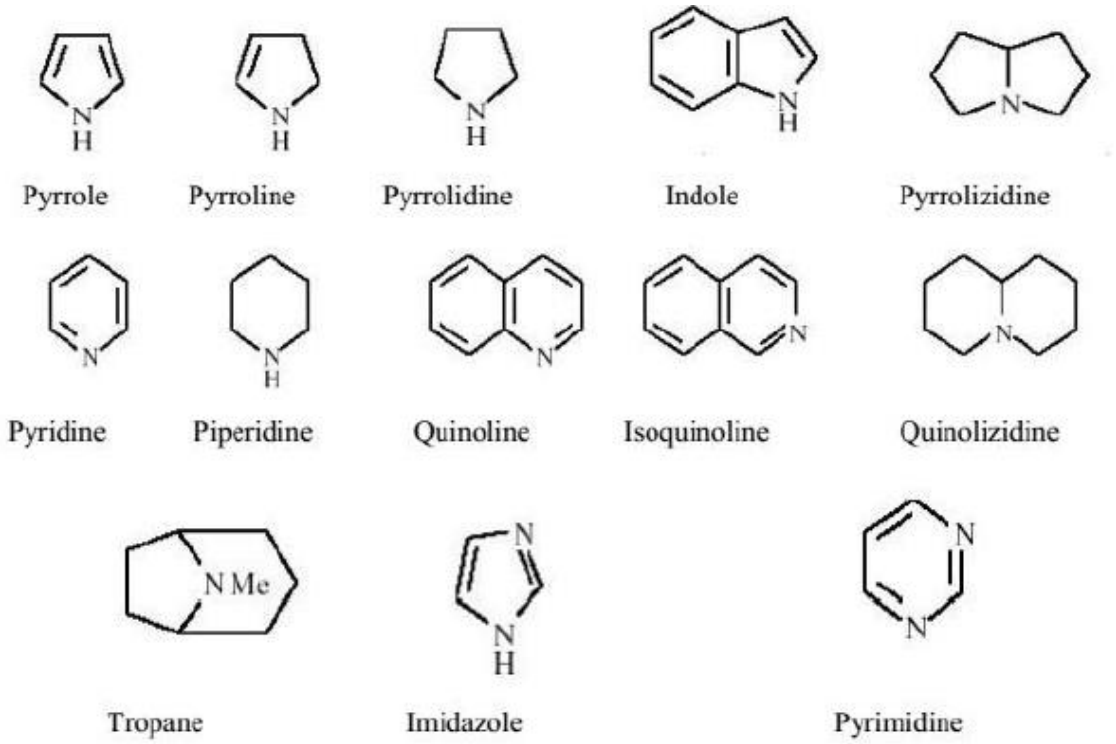

Fig1: Various types of alkaloids groups according to chemistry

Beginning with the first official pharmaceuticals, compendia monographs contained analytical assay procedures with the intention of characterizing the drug molecule. In recent years, titrimetric, spectrometric, chromatographic, electrophoresis, and other analytical methods have been used as assay methods in monographs. From the early stages of drug research to commercialization, an analytical approach is crucial. As a result, it is crucial in the field of pharmaceutical research to analyze any active component, intermediate, drug product, drug formulation, degradation product, and biological sample. The characterization of chemical constituents has greatly benefited from chemical degradation, transformation, and analyses by spectroscopic methods [8,9] when these analytical principles are taken into account.

Aim and Objectives

1. Analytical estimation and validation of amide alkaloids for development of new measurement techniques to offer better chemical data
2. The suggested study is utilized to develop proven routes for the development of new products and their evaluation.
3. To study the pharmacokinetic, therapeutic, and toxicological effects of new drug entities, an appropriate analytical method is needed.
4. The discovery, development, and production of pharmaceuticals depend heavily on the development and validation of analytical methods.
5. The validation's major goal is to show that the analytical method is accurate, specific, and exact and adequate for the task at hand.

Literature Review

- Toroto F.G. et.al. (1976) A novel piperine-type alkaloid called wisanine was discovered in the roots of the medicinal plant Piper guineenae (also known as West moan black or Ashanti Pepp) by Toroto F.G. et al. in 1976. This substance is the main component of the drug. (10)
- Souza VGD. Et. Al. (2017) suggested validating an analytical technique for quantifying quercetin and standardizing the hydroalcoholic extract (HAE) of A. colubrina using high-performance liquid chromatography with diode array detector (HPLC-DAD). In order to standardize extracts and ensure the quality of the herbal medicine containing the A. colubrinaPhyto complex, the procedure was verified using HPLC-DAD. (11)
- Kadam PV. ET AL. (2019) A crucial requirement to support natural goods' acceptability and activity is to design a quality assessment measure. A difficult task is developing valid and trustworthy analytical techniques that profile the quantitative phytochemical content of marker elements in multicomponent compositions like extract.. The suggested validation establishes the suitability of the HPLC-method for the analysis of ellagic acid from Canadian willow plant, extract, under specified circumstances. (12)
- Patel R.et al. (2016) researched the creation of reliable analytical techniques that can accurately predict the phytochemical composition, including quantitative studies of bioactive substances and other significant ingredients; Herbs are a complex system of combinations, making pharmacognostic study of them difficult. The most widely used techniques for ensuring the quality of raw materials and finished herbal products include developed analytical separation techniques such as ultra high performance liquid chromatography (UHPLC), gas

chromatography (GC), high performance thin layer chromatography (HPTLC), and hydrophilic interaction chromatography (HILIC). (13)

- Rakesh S. Shivatare et.al (2013) To address the complicated character of herbal medications, recommended analysis and quality control of herbal medicines. One of the sophisticated instrumental approaches for qualitative and quantitative examination of herbs and herbal medicines is high-performance thin layer chromatography (HPTLC). The creation of analytical methods based on HPTLC and the evaluation of validation characteristics are the main topics of this paper. (14)
- Balammal G et al. (2012 With careful consideration for already-in-place national legislation as well as national and regional norms, examined a collection of test procedures to help the formulation of national standards based on local market realities. (15)
- Batista ANL. Etal. (2011) A HPLC method was created and verified for the purpose of standardizing commercial and herbal extracts of Myrciauniflora. HPLC-PAD was used to determine the chromatographic profiles of M. unifloraleaves and commercial extracts. For the purposes of creating and validating the HPLC method, myricitrin was employed as an outside standard. (16)
- Mensah IA. et al.(1977) Wisanine and wisanidine, two novel amide alkaloids, were discovered to be N-piperidyl-5 (2-methoxy-4, 5-methylenedioxyphenyl)-trans-2-trans-4-pentadienamide and N-pyrrolidyl-5-(2-methoxy-4, 5-methylenedioxyphenyl)-trans-2-trans-4-pentadienamide, respectively, in the petroleum-extract of the roots of Pipe (17)

- Okwute SK. Et al. (1984) guineensine and wisanine, two pipcrolein acids that have been synthesized, were given with their updated structures. The &-isomer, known in the literature as the truns-isomer, was produced by UV irradiating transpiperolein B acid ester. Piperolein B aldehyde and 2-methoxy piperonal were reacted with the proper Wittig or Reformatsky reagent to produce the tram-2, trans-4 ethylenic linkages, which were then inserted into guineensine and wisanine. (18)
- Herbstein FH. Et al. (1981) The structure of the alkaloid wisanine was discovered (2-Methoxypiperine). The size and structure of the molecules are identical to those of 1-piperoylpiperidine (piperine). The investigation was done to compare the unsubstituted and substituted molecules' structural features. (19)
- Mgbeahuruike EE.et al.(2018**)** HPLC-DAD and UHPLC/Q-TOF MS analysis was undertaken to characterize and identify the chemicals present in the extracts with promising antibacterial activity. The piperamide alkaloids in the water extracts, which were nearly nonexistent, did not have any effect on the bacterial strains. Our findings show that P. guineense possesses antibacterial alkaloids that may be important for the research into the development of fresh natural antibiotics. (20)

Plan of Work

- Selection of plant
- Authentication of plant
- Separation of plant part
- Extraction of chemical constituents
- Isolation and purification of chemical constituents
- Stability of sample
- Development of the analytical method.
    a) Sample preparation for HPLC analysis
    b) HPLC Analysis
- Validation of analytical method
- Compilation of proposed research work

Methodology

The initial stage in separating the desired natural products from the raw ingredients is extraction. According to the extraction principle, there are several extraction procedures, including solvent extraction, distillation, pressing, and sublimation. The technique with the highest usage is solvent extraction. The following steps are taken when natural product extraction advances:

(1) the solvent permeates the solid matrix;

(2) The solute dissolves in the solvents

(3) the solute diffuses out of the solid matrix;

(4) The solutes that were extracted are gathered.(21)

The following is a succinct list of the numerous natural product extraction techniques:

a) Maceration

b)Percolation

c) Decotation-

i. Reflux extraction

ii. Soxhlet extraction

Extraction of amide alkaloids:

Following plant verification, the root portion was first dried and then broken up into smaller pieces for subsequent processing. Wisanine and wisanidine, two novel amide alkaloids, were isolated from the piper guineense dry root. Wisanine was isolated from an oily semi-solid that was deposited by the petroleum extract of the powdered root bark. It crystallizes as big yellow prisms from ethyl acetate. M.P. 179-181℃. Soxhlet apparatus was used to separate and structure wisanine from the stems and roots of Piper guineense. Two different groups have independently reported the synthesis of wisanine. (7,14)

The following study work is proposed to validate the HPLC method for quantification of samples from natural source extract in accordance with the ICH Guidelines.

HPLC Method Development and Validation: • Sample Acquisition.

a) Standard Test Method and preservation of chromatographic conditions

b) Mobile Phase Composition for HPLC

c) Preparation of Standard Solution of selected samples

d) Preparation of Test Solution

e) Analysis of Extract

f) Estimation of sample Present in Extract by HPLC

- **Validation Parameters:**

  The validation was carried out in accordance with recent ICH recommendations: Test were run on a number of parameters including ,

  a) Specificity
  b) Linearity
  c) Range
  d) Accuracy
  e) Precision
  f) Robustness
  g) Stability of solution

Conclusion and Future Prospective:

From the above proposed work, we conclude that the future development and analytical validation of amide alkaloid from natural source could be aninnovative and useful step for the development of a novel molecule for the various treatment and infections. The analytical estimation produced qualitative assay of procured extract which enhance the efficacy and safety profile of a new moiety. So the proposed work could produce a validated chemical compound with minimal toxic effects. The futuristic approach of the entitle work will produce the more effective analogue of plant extract towards in the biomedical applications.

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